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Factors discriminating men with coronary heart disease from healthy controls

Susan G. Cotton,[1] Janice M. Nixon, R. G. Carpenter, and D. W. Evans
From the Medical Statistics Section, Department of Human Ecology, University of Cambridge, and Addenbrooke's Hospital, Cambridge

In our study, 91 men, under 65 years of age, with clinically overt coronary heart disease were compared with 98 healthy men in respect of fasting plasma cholesterol, triglyceride and other lipid moieties, relevant past and family history, smoking habits, a ponderal index, blood pressure, and prevalence of corneal arcus, xanthomata, and baldness. Some dietary aspects were also taken into account.

The analysis showed that the main discriminators were diastolic blood pressure, arcus, baldness, xanthelasma, a family history of hypertension, past smoking habits, and hyperlipidaemia. Of these factors, diastolic hypertension emerges as much the most important.

Previous studies, and in particular those carried out in the U.S.A., have shown higher average serum or plasma cholesterol values in men with overt coronary heart disease than in control groups (Steiner and Domanski, 1943; Lawry *et al.*, 1957; Doyle *et al.*, 1957; Keys, 1967). The differences have usually been greatest at the younger ages. Some, however, have suggested better discrimination on the basis of serum or plasma triglyceride values (Albrink, Meigs, and Man, 1961; Hayes and Neill, 1964; Schilling, Becker, and Christakis, 1966) or some combination of the cholesterol and triglyceride figures (Butkus *et al.*, 1966; Rifkind, Lawson, and Gale, 1968).

Interest in the possibility of reducing the incidence of coronary heart disease by dietary modification (Page and Stamler, 1968; Dayton *et al.*, 1969) or other means of correcting hyperlipidaemia led us to seek evidence of this disorder in local patient and control populations. We therefore measured the fasting levels of various lipid moieties in the plasma of men under 65 years of age with overt coronary heart disease and compared them with these levels in a population of healthy men. We were also interested to compare the groups in respect of other relevant characteristics such as systolic and diastolic hypertension, past and present smoking habits, arcus senilis, baldness, body weight, and a history of gout. Detailed analysis of these data was undertaken with a view to identifying combinations of characteristics which might best describe the coronary heart disease subject.

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[1] Present address: Royal Infirmary, Cardiff.

Populations studied

The patients were men aged 35-64 years at the time of the study. Their names were obtained from the Addenbrooke's Hospital register of cases of acute myocardial infarction admitted under the care of two consultant physicians. Perusal of their records showed that all had suffered ischaemic cardiac pain, persisting for more than half an hour while at rest, between 4 months and 10 years before this study. In all but 3 cases there was good supportive evidence (electrocardiographic and enzyme changes) of myocardial infarction; for these 3 exceptions the electrocardiographic changes were minimal and non-specific and no abnormal SGOT value was recorded. Where there was evidence, in the records, of associated diabetes, nephrosis, or other disease known to affect plasma lipid levels, or where the patients were known to be taking oestrogens, corticosteroids, or antithyroid drugs, they were excluded from further consideration.

Permission to approach the remaining 148 patients was sought from their medical advisers and was refused in 16 cases – usually because of personality disorder or because of travelling difficulties. In another 17 instances the patients had moved away and 11 others had died since their last attendance at hospital. The remaining 104 patients were asked to attend for outpatient examination and withdrawal of a blood specimen after a 14-hour fast. Four declined to attend. Of the 100 patients ultimately seen, 9 had received some definite drug or dietary hypolipidaemic therapy and this was noted.

The control population was drawn from two panels of the Regional Blood Transfusion Service register of male donors under age 65 years. It was possible to select only the older donors from the second panel so that the ages of the control subjects roughly matched those of the patients, but there was no way of telling in advance of the interview to which social class the donor belonged. In response to letters explaining the nature and purpose of the investigation, 125 donors volunteered for interview and testing. Of these, 3 gave a history of angina and one of intermittent claudication; these, and one who proved to be frankly myxoedematous, were excluded from the study. Of the remaining 120 donors, data from 22 aged less than 35 years were excluded from the analysis as there were no patients of comparable age. The utilized control group thus numbered 98.

Methods

Clinical assessment Data obtained included social class (from the General Register Office Classification of Occupations), tea and coffee consumption (as an index of caffeine intake), egg consumption, personal past history of diabetes, hypertension, renal or biliary disease or gout, family history of diabetes, coronary thrombosis and sudden death, stroke, hypertension or hyperlipidaemia, details of any personal history of angina or intermittent claudication. Past and present smoking habits were recorded and converted to equivalent cigarettes per day as described by Fairbairn, Wood, and Fletcher (1959). For the patients only, a limited dietary history was also obtained and analysed, an attempt being made to determine any change in their dietary habits since the coronary attack.

Height (without shoes) and weight (in trousers and underclothes) were recorded for each subject. For the patients, their first weight in hospital during the coronary episode was also noted so that any subsequent weight change could be estimated. Blood pressure was measured sphygmomanometrically by the same observer in every case, the subject lying supine and having rested thus for about 5 minutes before the readings were taken. A search was made for xanthelasma, tendon and cutaneous xanthomata. Presence or absence of corneal arcus was also noted; where present this was graded 1 to 3 on the basis of less than a semicircle, more than a semicircle but less than a complete ring, and a complete ring, respectively. Any degree of baldness was graded 1 to 3 according to its appearance as receding frontal hairline, a vertical bald area, or total or subtotal hair loss (i.e. side tufts, only, remaining). All the patients, but not the control subjects, underwent full clinical examination of their cardiovascular and respiratory systems and of other systems if symptoms warranted. At the conclusion of the examination and documentation, and having checked that the 14-hour fast had been observed, 20 ml venous blood was taken from each subject (patients and controls). This sample was divided into two portions, one being sent in a sequestrene container for plasma cholesterol and triglyceride estimation and the other being anticoagulated with heparin before lipoprotein, phospholipid, and free fatty acid assay.

Laboratory methods Plasma cholesterol was measured by an autoanalyser technique based on the reagent of Huang *et al.* (1961). Values obtained by this technique averaged 6·5 mg per dl lower than the values obtained by the same laboratory's non-automated method (Jamieson, 1964) used hitherto. The mean plasma cholesterol by the automated technique in a population of 97 healthy men aged 17–24 years studied in February 1970 was 165 mg per dl (SD 24 mg per dl).

Plasma triglyceride was also measured by autoanalyser, the method being basically that of Löfland (1964). Because of the requirement for samples from fasting subjects, a 'normal range' has not yet been determined by this technique.

Total phospholipid was measured by assaying lipid phosphorus according to the method of Allen (1940) after methanol-chloroform extraction from plasma (Folch, Lees, and Sloane-Stanley, 1957). Free fatty acid concentration was measured by Dole's (1956) extraction method.

Separation of lipoproteins was by electrophoresis on cellulose acetate using Sephraphor III strips (Gelman Instrument Co., U.S.A.) in 0·00238 M Gelman High Resolution buffer, *p*H 8·8. Runs were made at room temperature for 85 minutes at 325 V with a current of 1·4 mA per strip. The strips were stained according to the ozone-Schiff's method of Kohn (1961) and examined by reflected light in a Chromoscan (Joyce Loebl Instruments, England). The lipoprotein concentrations were then determined according to the method of Magnani and Howard (1971) and expressed in terms of their preparative ultracentrifuge fractionation equivalents, i.e. as 'high-density lipoprotein' (HDL), 'low-density lipoprotein' (LDL), and 'very-low-density lipoprotein' (VLDL).

Data processing The clinical and biochemical data were transferred to punched cards for computer analysis. In doing so, for patients known to be receiving hypotensive therapy at the time of the survey examination (13 cases), pretreatment blood pressure readings were obtained from hospital records and used throughout the analysis. This step was considered desirable, and justifiable in so far as the technique of blood pressure measurement was essentially unchanged. It was not possible similarly to substitute serum cholesterol levels obtained before beginning hypolipidaemic therapy in the 9 patients on such treatment, because the method for cholesterol assay used during the survey was substantially different from that used previously, and plasma levels taken within a few weeks of acute myocardial infarction are not necessarily representative of subsequent stable levels (Cotton, 1970). Accordingly, data from these 9 subjects were excluded from the analysis. The effects of these exclusions were investigated.

Three derived variables were added:

i) HDL/LDL ratio.

ii) A ponderal index $= \frac{\text{Weight}}{\text{Height}^2} \times 100$.

This formula was preferred because it is uncorrelated with height (Khosla and Lowe, 1967). Ponderal index was calculated for all subjects using their present weight and for the patients only using their past weight also.

iii) 'Hyperlipidaemia' was arbitrarily scored as follows.

Hyperlipidaemia score	*Cholesterol mg/dl*	*Triglyceride mg/dl*
0	<250	All values
1	$\geqslant 250$	<140
2	$\geqslant 250$	$\geqslant 140$

TABLE I *Results of single variable analysis on qualitative variables*

	Present in		*Difference of (%)*	*Significance level*†
	Patients (%)	*Controls (%)*		
Smoking now	61·5	54·1	7·4	NS
Smoked past	96·7	85·7	11·0	**
Past history diabetes	0·0	0·0	—	—
Past history hypertension	18·7	1·0	17·7	***
Past history renal disease	12·1	6·1	6·0	NS
Past history biliary disease	4·4	2·1	2·3	NS
Past history gout	6·6	1·0	5·6	NS
Family history diabetes	13·2	15·3	−2·1	NS
Family history hypertension	15·4	19·4	−4·0	NS
Family history stroke	26·4	28·6	−2·2	NS
Family history coronary disease	41·8	26·5	15·3	*
Family history hyperlipidaemia	0·0	0·0	—	—
Xanthelasma	3·3	0·0	3·3	NS

† Significance levels obtained by χ^2 tests are indicated as follows:
NS Not significant;
* $0·01 < P \leqslant 0·05$;
** $0·001 < P \leqslant 0·01$;
*** $P \leqslant 0·001$.

Results

Single variable analysis The results of single variable analysis of qualitative variables are shown in Table 1 and of quantitative and graded variables in Table 2. Tables 1 and 2 show statistically significant differences between patients and controls on social class, height, diastolic blood pressure, systolic blood pressure, arcus, baldness, plasma cholesterol, 'hyperlipidaemia', previous smoking habits, history of hypertension, and family history of coronary disease. Other differences are not significant.

The results obtained from the limited dietary history did not reveal any significant relation between fat consumption and plasma lipids in the patient group.

Multivariate comparison Because the variables may be correlated, discriminant analysis was necessary to determine the essential differences between cases and controls. This was done using a computer programme BMDO2R (Dixon, 1965), and it was necessary to exclude a further 7 patients and 7 controls because of incomplete biochemical data, thus leaving 84 patients and 91 controls.

The composite variables HDL/LDL ratio and ponderal index were excluded from this analysis as they had shown no significant difference between the patients and controls, and it seemed unlikely that they would add any information to that contained in the individual variables of which they were composed. Of the information collected under past history and family history, only a past history of hypertension and a family history of hypertension, stroke, and coronary disease were included as likely to be relevant. The quantity smoked now was also excluded as most of the patients had been advised to reduce the amount that they smoked.

Age and social class were treated as covariables so that patients and controls could be regarded as comparable in this respect. Methods for handling covariables in discriminant analysis have been discussed by Cochran and Bliss (1948).

The results of the analysis showed that the main discriminators between the two groups in descending order of observed importance were diastolic blood pressure, presence of arcus, degree of baldness, presence of xanthelasma, a family history of hypertension, quantity smoked in the past, and plasma cholesterol, triglyceride, and LDL. Xanthelasma (the only form of xanthoma found) is significant because the only three examples seen were in patients. It was found desirable to include weight (present) in the discriminant function as this increased the effect of triglyceride. The combined variable 'hyperlipidaemia' included the information contained in the variables cholesterol and triglyceride and could be used to replace them. When this was done the effect of LDL, which appeared to be linked to cholesterol and triglyceride, was reduced to insignificance.

To make the discriminant coefficients comparable, we have used standardized variables, i.e. each variable divided by its standard deviation, for presenting the results. The coefficients of standardized variables for the discriminant function are given with their standard errors and significance levels in Table 3.

Discriminant scores were obtained from

TABLE 2 *Results of single variable analysis on quantitative variables*

	Means		*Pooled standard deviation*	*Difference of means*	*SE of difference*	*Significance level**
	Patients	*Controls*				
Age (yr)	54·14	52·65	7·027	1·49	1·023	NS
Social class	3·09	2·35	0·891	0·74	0·130	***
Quantity smoked now (g/day)	5·84	7·05	8·982	−1·21	1·308	NS
Quantity smoked past (g/day)	20·28	16·08	12·884	4·20	1·876	*
Eggs eaten per week	4·54	5·28	3·379	−0·74	0·493	NS
Caffeine consumption (cups/day)	7·31	7·74	3·031	−0·43	0·441	NS
Height (in)	67·32	68·26	2·693	−0·94	0·392	*
Past weight (lb)	165·31	168·42†	21·871	−3·11	3·193	NS
Present weight (lb)	163·48	168·42	20·418	−4·94	2·972	NS
Diastolic blood pressure	93·24	81·28	13·261	11·96	1·930	***
Systolic blood pressure	150·33	134·08	25·232	16·25	3·673	***
Arcus	0·89	0·44	0·939	0·45	0·137	***
Baldness	1·10	0·69	0·784	0·41	0·114	***
Cholesterol	269·87	252·20	40·993	17·67	5·968	**
Triglyceride	174·69	152·23	87·230	22·46	12·939	NS
Free fatty acid	452·87	480·17	137·154	−27·30	20·203	NS
Phospholipid	240·57	234·26	39·441	6·31	5·756	NS
HDL	271·06	272·24	74·937	−1·18	11·204	NS
VLDL	147·10	124·95	124·371	22·15	18·594	NS
LDL	400·24	383·00	111·358	17·24	16·649	NS
Total lipoprotein	813·30	780·51	241·939	32·79	36·172	NS
HDL/LDL	0·71	0·74	0·228	−0·03	0·034	NS
Ponderal index using past weight (lb/in^2)	3·66	3·61†	0·427	0·05	0·062	NS
Ponderal index using present weight (lb/in^2)	3·61	3·61	0·402	0·00	0·059	NS
'Hyperlipidaemia'	1·13	0·74	0·844	0·39	0·128	**

* The significance levels obtained from Student's t test are indicated as in Table 1.
† The past weight of the controls was assumed to be the same as their present weight.

each subject's unstandardized measurements as follows—

$$\begin{aligned}\text{Score} = & -6{\cdot}386 \times (\text{Social Class}^1 - 2{\cdot}71)\\ & -1{\cdot}264 \times (\text{Age in years} - 53{\cdot}32)\\ & +0{\cdot}584 \times \text{Quantity Smoked Past (g/day)}\\ & -19{\cdot}113 \times \text{Family History of Hypertension } (0=\text{No}, 1=\text{Yes})\\ & +1{\cdot}316 \times \text{Diastolic Blood Pressure (mmHg)}\\ & -0{\cdot}228 \times \text{Weight (lb)}\\ & +12{\cdot}736 \times \text{Arcus}^1\\ & +11{\cdot}834 \times \text{Baldness}^1\\ & +63{\cdot}523 \times \text{Xanthelasma } (0=\text{No}, 1=\text{Yes})\\ & +7{\cdot}767 \times \text{Hyperlipidaemia}^1\\ & -112{\cdot}696.\end{aligned}$$

The first two terms relating to social class and age adjust the score for differences between the subjects in these variables. When studying subjects of a given age and social class these terms may be omitted.

Fig. 1 shows histograms of these discriminant scores for patients and controls.

Discussion

The most interesting finding, suggested by the difference in the means shown in Table 2, is that diastolic blood pressure appears more closely related to overt coronary heart disease than plasma lipid levels. Table 3 shows that the discriminant coefficient for diastolic blood pressure measured in standardized units is 18·97 ± 3·32 compared to 6·71 ± 3·21 for the combined variable 'hyperlipidaemia' in standardized units, and the analysis showed the latter variable to contain all the discriminating

[1] The variables are classified as defined in the text.

TABLE 3 *Coefficients with standard errors for the discriminant function and standard deviations used to standardize variables*

Variable	*SD for standardization*	*Discriminant coefficient and SE of standardized variable*		*Significance level**
		Coefficients	*SE*	
Diastolic blood pressure	14·415	18·97	3·32	***
Arcus	0·954	12·15	3·34	***
Baldness	0·810	9·58	3·24	**
Xanthelasma	0·130	8·27	3·18	**
Family history hypertension	0·388	−7·41	3·18	*
Quantity smoked past	12·562	7·34	3·19	*
Hyperlipidaemia	0·864	6·71	3·21	*
Weight	20·695	−4·73	3·18	NS

* The significance levels are as defined for Table 1.
The variables are assumed to be in standardized units and the scale has been chosen so that the discriminant scores range from −100 to +100 approximately.

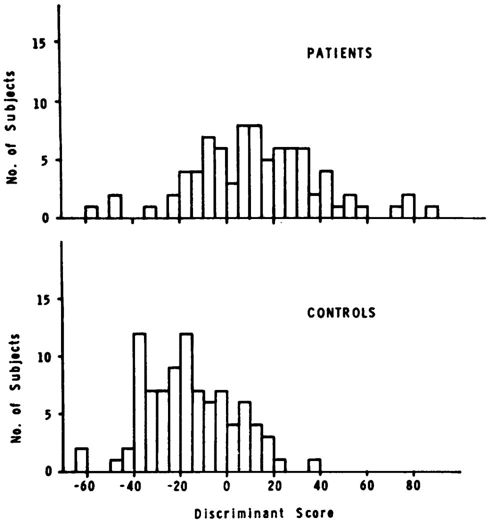


FIG. 1 *The discriminant scores of patients and controls, derived from the multivariate discriminant function.*

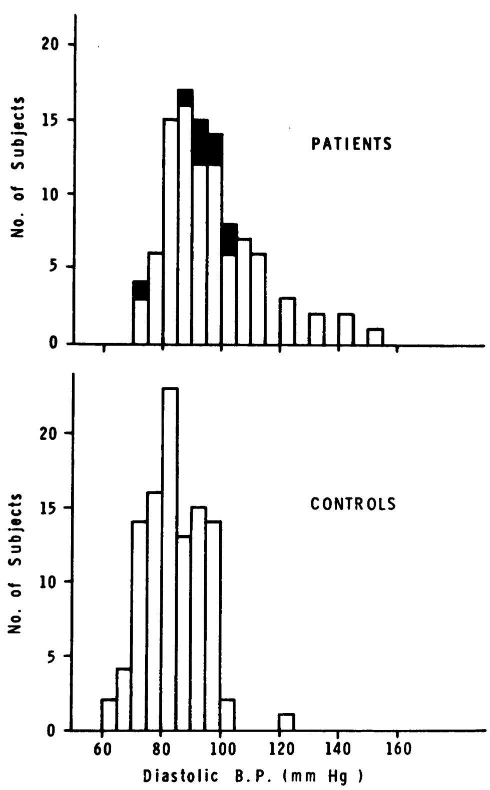
information in the plasma lipid levels examined. Taking the negative correlation between the coefficients into account, the difference between them is statistically significant, $0.01 < P \leq 0.05$. This finding implies that for any given level of plasma cholesterol and triglyceride, a raised blood pressure is more likely to be associated with coronary heart disease than is a corresponding rise in lipid levels when blood pressure is held constant.

Weight loss frequently occurs in patients after myocardial infarction and could result in reduction in cholesterol levels measured subsequently. However, this factor is unlikely to have been material to our findings because there was a mean weight difference of less than 2 lb between present and past weights in this group of patients.

The effect of excluding patients on hypolipidaemic therapy was investigated as follows. Pretreatment cholesterol levels were used for these patients and the corresponding triglyceride levels estimated from the regression relation between these two variables found from the rest of our data. We suspect that the cholesterol levels, and hence both levels, may have been inflated by the technical methods in use at the time of these earlier estimations. The regression relation was also used to obtain triglyceride values for the 7 patients and 7 controls who had been excluded from the multivariate analysis because of missing data. The discriminant analysis was then repeated using all the patients and controls, substituting estimated lipid levels where necessary. It was found that the coefficient of diastolic blood pressure was still statistically significantly larger than that of 'hyperlipidaemia' ($0.01 < P \leq 0.05$), both variables being measured in standardized units. The revised coefficients for those variables are 19.18 ± 3.05 and 9.14 ± 2.96, which may be compared with the previous values of 18.98 and 6.71. Regression coefficients of other variables are not greatly altered. Thus we conclude that initial exclusion of the 9 patients on hypolipidaemic therapy probably led to some underestimation of the importance of 'hyperlipidaemia', but not so much as to warrant modifying our conclusions as to its importance in relation to hypertension.

Fig. 2 shows the distributions of diastolic blood pressure of patients and controls and Fig. 3 shows the distributions of plasma cholesterol levels; the values contributed by the 9 patients on hypolipidaemic therapy are indicated by the shaded areas. The relative discriminating power of blood pressure and

FIG. 2 *The number of patients and controls by diastolic blood pressure. Shaded areas are current blood pressure values of patients on hypolipidaemic therapy.*



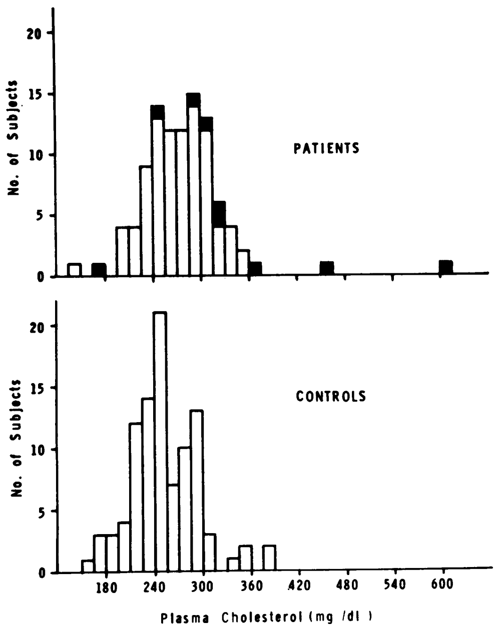


FIG. 3 *The number of patients and controls by plasma cholesterol level. Shaded areas are pretreatment cholesterol values of patients on hypolipidaemic therapy.*

cholesterol (the principal factor in 'hyperlipidaemia') may be judged from Fig. 2 and 3.

In common with other investigators, we found a greater tendency towards hypercholesterolaemia in the younger patients (35–44 years). The same tendency appeared to exist in respect of low-density lipoprotein levels, as noted by Gofman, Young, and Tandy (1966).

The dietary analysis revealed no relation between plasma cholesterol levels of patients and their habitual consumption of fat (or proportion of unsaturated-fatty-acid fat), eggs, or carbohydrate. This finding accords with the results of a recently published similar inquiry (Framingham Study, 1970) but runs counter to pooled international experience including data from large-scale population studies (Fejfar, 1970). This discordance may be explained by the possibility that dietary fat variation about a relatively high mean intake may exert comparatively little influence on the associated relatively high plasma cholesterol levels.

Our data showed corneal arcus to be a useful discriminator between the coronary heart disease patients and the control population, second in importance only to hypertension (of which it proved independent). This was principally so in the age group 45–54 years, a finding compatible with that of Rifkind (1965). Unlike Rifkind, however, and in accordance with the findings of Hickey, Maurer, and Mulcahy (1970), we could not show any correlation between presence or grade of arcus and plasma cholesterol level. The mechanism of this relation between arcus and coronary heart disease is obscure. There are certainly racial factors involved in its development (Cook and Kanyerezi, 1970) and there may well be different types of arcus depending on the nature of the circulating lipids and target-organ sensitivity. However, the possibility remains that the presence of arcus in young or middle-aged Caucasian men may indicate the earlier existence of raised plasma lipid levels over a substantial period of time. Alternatively, it may indicate the presence of increased membrane permeability to lipids, or subnormal clearance capability, which might apply also to the arterial intima. In either case there could be a link with the development of coronary disease.

Rather surprisingly, baldness also proved a good discriminator between patients and controls. It showed no significant correlation with any other variable recorded. Past smoking habits similarly discriminated between patients and controls, the percentage who smoked and the amount smoked in the past being significantly greater in the patient group. There was no significant difference in current smoking habits of the two groups and this probably reflects the fact that some of the patients gave up smoking or reduced the amount smoked after their coronary attacks.

The relative unimportance of plasma cholesterol in discriminating between patients and controls was an unexpected finding of this study. However, both populations were hypercholesterolaemic by comparison with a group of normal, young, adult men studied with the same technique, and by reference to world figures for normal serum or plasma cholesterol (Reisel, 1968). It is therefore arguable that both populations were at risk in regard to their plasma lipid status, the degree of this risk being not very dissimilar. Also, this risk may operate particularly in the long-term sense and in respect of the cryptic stage of coronary disease. If this is so there are seen to be difficulties regarding the representative value of solitary plasma cholesterol levels and the unknown incidence of subclinical coronary disease in the two groups. Nevertheless, hypertension has emerged as the dominant factor in the Framingham prospective study (1969) also and it may be concluded that raised blood pressure, perhaps acting over a shorter period than hypercholesterolaemia, has a greater influence in determining the onset of clinically overt coronary heart disease than a rise of the plasma cholesterol above the levels generally prevailing.

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References

Albrink, M. J., Meigs, J. W., and Man, E. B. (1961). Serum lipids, hypertension and coronary artery disease. *American Journal of Medicine*, **31**, 4.

Allen, R. J. L. (1940). The estimation of phosphorus. *Biochemical Journal*, **34**, 858.

Butkus, A., Berrettoni, J. N., Sones, F. M., Jr., Noto, T. J., and Page, I. H. (1966). Double-blind study of serum lipids in patients who have had coronary angiography. *Circulation*, **33** and **34**, Suppl. III, 68.

Cochran, W. G., and Bliss, C. I. (1948). Discriminant functions with covariance. *Annals of Mathematical Statistics*, **19**, 151.

Cook, G. C., and Kanyerezi, B. R. (1970). Corneal arcus in the Ugandan African. *American Heart Journal*, **79**, 718.

Cotton, S. G. (1970). Plasma cholesterol after myocardial infarction. *Postgraduate Medical Journal*, **46**, 551.

Dayton, S., Pearce, M. L., Hashimoto, S., Dixon, W. J., and Tomiyasu, U. (1969). A controlled clinical trial of a diet high in unsaturated fat in preventing complications of atherosclerosis. *Circulation*, **40**, Suppl. II.

Dixon, W. J. (1965). BMD Biomedical Computer Programs. University of California, Los Angeles.

Dole, V. P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *Journal of Clinical Investigation*, **35**, 150.

Doyle, J. T., DeLalla, L. S., Baker, W. H., Heslin, A. S., and Brown, R. K. (1957). Serum lipoproteins in preclinical and in manifest ischaemic heart disease. *Journal of Chronic Diseases*, **6**, 33.

Fairbairn, A. S., Wood, C. H., and Fletcher, C. M. (1959). Variability in answers to a questionnaire on respiratory symptoms. *British Journal of Preventive and Social Medicine*, **13**, 175.

Fejfar, S. (1970). Communication to VIth World Congress of Cardiology, London.

Folch, J., Lees, M., and Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, **226**, 497.

(The) Framingham Study, an Epidemiological Investigation of Cardiovascular Disease (1969). Ed. by W. B. Kannel and T. Gordon. U.S. Government Printing Office, Washington, Section 23.

(The) Framingham Study (1970). Ed. by W. B. Kannel and T. Gordon. U.S. Government Printing Office, Washington, Section 24.

Gofman, J. W., Young, W., and Tandy, R. (1966). Ischaemic heart disease, atherosclerosis, and longevity. *Circulation*, **34**, 679.

Hayes, D., and Neill, D. W. (1964). Serum cholesterol and triglycerides in ischaemic heart disease. *Clinical Science*, **26**, 185.

Hickey, N., Maurer, B., and Mulcahy, R. (1970). Arcus senilis: its relation to certain attributes and risk factors in patients with coronary heart disease. *British Heart Journal*, **32**, 449.

Huang, T. C., Chen, C. P., Wefler, V., and Raftery, A. (1961). A stable reagent for the Liebermann-Burchard reaction. Application to rapid serum cholesterol determination. *Analytical Chemistry*, **33**, 1405.

Jamieson, A. (1964). A method for the rapid determination of serum total cholesterol using a modification of the Pearson reaction. *Clinica Chimica Acta*, **10**, 530.

Keys, A. (1967). *Arteriosclerosis*, 2nd ed., p. 579. Ed. by E. V. Cowdry. Charles C. Thomas, Springfield, Illinois.

Khosla, T., and Lowe, C. R. (1967). Indices of obesity derived from body weight and height. *British Journal of Preventive and Social Medicine*, **21**, 122.

Kohn, J. (1961). A lipoprotein staining method for zone electrophoresis. *Nature (London)*, **189**, 312.

Lawry, E. Y., Mann, G. V., Peterson, A., Wysocki, A. P., O'Connell, R., and Stare, F. J. (1957). Cholesterol and beta-lipoproteins in the serum of Americans; well persons and those with coronary heart disease. *American Journal of Medicine*, **22**, 605.

Löfland, H. B., Jr. (1964). A semiautomated procedure for the determination of triglycerides in serum. *Analytical Biochemistry*, **9**, 393.

Magnani, H. M., and Howard, A. N. (1971). A quantitative method for blood lipoproteins using cellulose acetate electrophoresis. *Journal of Clinical Pathology*, **24**, 837.

Page, I. H., and Stamler, J. (1968). Diet and coronary heart disease. *Modern Concepts of Cardiovascular Disease*, **37**, 119.

Reisel, J. H. (1968). *Hypercholesterolaemia and Clinical Atherosclerosis*, p. 46. Wyt, Rotterdam.

Rifkind, B. M. (1965). The incidence of arcus senilis in ischaemic heart-disease and its relation to serum-lipid levels. *Lancet*, **1**, 312.

Rifkind, B. M., Lawson, D., and Gale, M. (1968). Diagnostic value of serum lipids and frequency of lipoprotein patterns in myocardial infarction. *Journal of Atherosclerosis Research*, **8**, 167.

Schilling, F. J., Becker, W. H., and Christakis, G. (1966). Serum cholesterol and triglyceride in 2,100 men and women, normal and high-risk coronary. *Circulation*, **33** and **34**, Suppl. III, 28.

Steiner, A., and Domanski, B. (1943). Serum cholesterol level in coronary arteriosclerosis. *Archives of Internal Medicine*, **71**, 397.

Requests for reprints to Dr. David W. Evans, Addenbrooke's Hospital, Trumpington Street, Cambridge, CB2 1QE.